ELSEVIER

Rhizosphere colonization of hexaploid wheat by *Pseudomonas fluorescens* strains Q8r1-96 and Q2-87 is cultivar-variable and associated with changes in gross root morphology

Patricia A. Okubara,[a],* Jeremy P. Kornoely,[a] and Blanca B. Landa[b]

[a] *US Department of Agriculture, Agricultural Research Service, Washington State University, Pullman, WA, USA*
[b] *Departamento de Agronomía, Escuela Técnica Superior de Ingenieros, Agrónomos y de Montes (ETSIAM), Universidad de Córdoba, Apartado 3048, Córdoba 14080, Spain*

Abstract

In the Pacific Northwest, natural suppression of take-all disease of wheat (take-all decline) has been attributed to certain strains of the gram-negative bacterium *Pseudomonas fluorescens*. To examine naturally occurring variation in the ability of *Triticum aestivum* L. (hexaploid wheat) to support these bacteria, we have surveyed 27 Pacific Northwest (PNW) cultivars for the ability to undergo root colonization with the aggressive colonizer *P. fluorescens* strain Q8r1-96, and *P. fluorescens* strain Q2-87, a less effective colonizer. In seed inoculation experiments, Q8r1-86 colonized roots of all of the cultivars equally or more effectively than did Q2-87 in a non-pasteurized, non-agricultural soil. Seven cultivars supported significantly ($P < 0.05$) higher rhizosphere populations of Q8r1-96 than Q2-87 within 14 days post-inoculation (dpi), two cultivars supported relatively high population densities of each bacterial strain, and three cultivars supported low population densities of the strains. Population densities normalized to root weight reached maximum steady-state levels within 4 dpi, and differential colonization was seen as early as 7 dpi. In pairwise comparisons, the bacterial treatments differentially affected the root morphology of some of the cultivars at 14 dpi. However, principal components (factor) and correlation analysis showed that preferential colonization by Q8r1-96 was independent of root fresh weight, total length, surface area, volume, and average diameter, and that differential colonization was not correlated with changes in any specific root morphometric variable. Variation in root colonization of specific cultivars suggests useful genetic stocks for mapping and identifying host genes involved in wheat–rhizosphere interactions.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Biological control; *Triticum aestivum*

1. Introduction

The practice of direct seeding, or sowing of seed into the stubble of the previous crop, has increased in the US since the mid-1990s. The benefits of direct seeding, specifically topsoil conservation and reduction of input costs, can be offset by the increased incidence and severity of fungal diseases. Increased disease severity has been attributed in part to the build-up of pathogen populations on wintering stubble (Cook et al., 2000; Windels, 2000). *Triticum aestivum* L. (hexaploid wheat) and related cereal species have limited genetic resistance

to necrotrophs such as *Gaeumannomyces tritici* var. *graminis*, *Rhizoctonia solani*, and *Pythium* spp. that are among the major soilborne pathogens of small grain cereals in the Pacific Northwest. Furthermore, transferable sources of resistance are rare (Smith et al., 2003a,b). Plant growth-promoting rhizobacteria (PGPR) can be highly effective in the suppression of these diseases (Becker and Cook, 1988; Kim et al., 1997; Weller and Cook, 1983, 1986), and offer an alternative supportive of sustainable agriculture.

In the Pacific Northwest, naturally occurring suppression of *G. graminis* var. *tritici* (*Ggt*), which causes take-all disease of wheat, is attributed to certain strains of *Pseudomonas fluorescens* that produce the antifungal metabolite 2,4-diacetylphloroglucinol (2,4-DAPG)

* Corresponding author. Fax: 1-509-335-7674.
E-mail address: pokubara@wsu.edu (P.A. Okubara).

(Weller et al., 2002). However, strains that produce 2,4-DAPG are genotypically and phenotypically diverse, and differ in efficacy against root fungal pathogens. *P. fluorescens* strain Q8r1-96 (Raaijmakers and Weller, 1998) is representative of a genotypic class (the D genotype) of 2,4-DAPG producers that is dominant in take-all suppressive soils (McSpadden Gardener et al., 2000; Raaijmakers and Weller, 2001). Members of the D genotype are substantially more competitive in the wheat rhizosphere than strains of most other genotypes of 2,4-DAPG producers such as Q2-87 (B genotype) (Landa et al., 2003; McSpadden Gardener et al., 2000; Weller et al., 2002). For example, strain Q8r1-96 typically maintains populations 100- to 1000-fold greater, and persists longer in the wheat rhizosphere, than does strain Q2-87 (Landa et al., 2003; Raaijmakers and Weller, 2001; Raaijmakers et al., 1997). The basis for the superior colonizing ability of Q8r1-96 is not fully understood. However, in suppressive subtractive hybridization experiments by Mavrodi et al. (2002), a number of genomic DNA segments unique to Q8r1-96 were found that might contribute to the rhizosphere competence of this strain.

The production of 2,4-DAPG in planta has been shown to be directly proportional to rhizosphere population density (Raaijmakers et al., 1999). Although soil type can influence the composition of fluorescent pseudomonads in the rhizosphere (Latour et al., 1996), the action of aggressive colonizers appears to be independent of soil type. For example, preferential colonization by Q8r1-96 as compared to Q2-87 was observed in three different natural, virgin Washington soils (Raaijmakers and Weller, 2001), and the growth promoting activity of strain F113 on pea roots was similar in three types of agricultural soil (De Leij et al., 2002). Population densities of 10^5 CFU g^{-1} are sufficient to control *Ggt* in non-agricultural soils (Weller et al., 2002).

To date, our understanding of rhizosphere interactions has been derived primarily from the role of the microbial partner. However, host traits such as root growth rate and cellular regeneration have been correlated with tolerance to root pathogens (Liljeroth, 1995; Scott, 1981). Sites of lateral root emergence have been postulated to serve as nutrient sources for rhizosphere microbes, and the quality and quantity of root exudates are also likely to impact the biotic composition of the rhizosphere (Hawes et al., 1998; Rengel, 2002). Such traits might be important in the wheat–*P. fluorescens* interaction.

Host species has been found to be another significant variable in PGPR-associated processes, including the biosynthesis of 2,4-DAPG in the rhizospheres of monocots and dicots as monitored by *phlA::lacZ* (Notz et al., 2001), and the extent of induction of systemic resistance in *Arabidopsis* (Van Wees et al., 1997). High population densities of *P. fluorescens* F113 were maintained in the rhizosphere of wheat but not pea, whereas isolate MVP1-4 was preferentially associated with the pea rhizosphere (Landa et al., 2002b).

Host variation in the ability to undergo PGPR interactions has been further observed at the cultivar level, as in the case of root colonization in cotton (Adams and Kloepper, 2002) and tomato (Smith and Goodman, 1999; Simon et al., 2001). In the latter case, three quantitative trait loci governing the suppression of *Pythium torulosum* by *Bacillus cereus* strain UW85 were genetically mapped (Smith et al., 1999). The authors noted that disease suppression co-segregated with at least two host traits, growth of UW85 on seeds and rate of seedling emergence, indicating the involvement of more than one host mechanism. We have found that PNW wheat cultivar Penawawa but not cv. Madsen supported higher population densities of Q8r1-96 than Q2-87 in sequential planting (cycling) experiments using inoculated soils (Okubara and Landa, unpublished data). Here, we extend our study to 27 PNW cultivars representing six market classes, and examine possible correlations between root colonization and quantitative root morphometric variables.

2. Materials and methods

2.1. Cultivars, bacterial inocula, and seed treatments

Seeds of 27 PNW wheat cultivars were treated with Rif$^+$ strains of the biological control bacterium *P. fluorescens*, Q2-87 or Q8r1-96, then roots were assayed for populations of Rif$^+$ bacteria at 4, 7, and 14 days post-inoculation (dpi). The PNW cultivars were selected on the basis of growth habit and end use (market class), acreage in 2001, flour quality traits, and resistance to leaf and stem pathogens (Table 1). Penawawa served as a standard cultivar in our seed treatment experiments because of its extensive use in serial colonization (cycling) studies (e.g., Landa et al., 2003; Raaijmakers and Weller, 2001), in which population densities of Q8r1-96 were observed to be significantly ($P < 0.05$) higher than those of Q2-87 after cycling. Seeds were obtained from the most recently harvested lots, specifically late summer/early fall of 2001 (14-day studies) and late summer 2002 (4- and 7-day studies).

Bacterial strains Q8r1-96 and Q2-87 were isolated from soil in Quincy, WA that was found to be naturally suppressive to the take-all pathogen, *Ggt*. Spontaneous rifampicin-resistant (Rif$^+$) strains were selected for use in root population density studies (Raaijmakers et al., 1999), and fully retained suppressiveness against take-all (Raaijmakers and Weller, 2001). The strains were cultured on 1:3 dilution of King's Media B (1/3 KMB) agar supplemented with 40 µg/ml ampicillin, 13 µg/ml chloramphenicol, 100 µg/ml cycloheximide, and 100 µg/ml rifampicin as de-

Table 1
Varieties of hexaploid wheat used in root colonization studies

Cultivar	Abbreviation	Market class[a]	Acreage rank[b]	Flour quality[c]	Resistance[d]
Alpowa	Alp	SWS	1	+	LR,YR
Boundary	Bou	HRW	8	+	DB,LR,PM,YR
Bruehl	Bru	SWWC	1	+++	SM,YR
Buchanan	Buc	HRW	3	+	CB,FS,SM
Chukar	Chu	SWWC	nd	+++	PM,SFR,YR
Coda	Cod	SWWC	3	++	LR,PM,SFR,SR,YR
Edwin	Edw	SWWC	2	++	SM,YR
Eltan	Elt	SWW	1	+	CB,DB,SM,YR
Finch	Fin	SWW	nd	++	PM,SFR,YR
Finley	Fnl	HRW	2	++	YR
Hatton	Hat	HRW	1	+	FS
Hiller	Hil	SWWC	6	+++	CB,YR
ID377S	ID3	HWS	1	+/−	YR
Lambert	Lam	SWW	7	+/−	LR,YR
Macon	Mac	HWS	nd	++	HF,YR
Madsen	Mad	SWW	2	+/−	CB,DB,LR,SR,SFR,YR
Penawawa	Pen	SWS	9	+/−	LR
Rely	Rel	SWWC	4	+++	LR,YR
Residence	Res	HRW	nd	−	nd
Rod	Rod	SWW	9	+	YR
Scarlet	Sca	HRS	1	++	LR,YR
Semper	Sem	HRW	nd	−	nd
Stephens	Ste	SWW	4	+	YR
Tara	Tar	HRS	nd	+++	HF,LR,YR
Tubbs[e]	Tub	SWW	nd	+	nd
Vanna	Van	SWS	10	+++	nd
Westbred926	WB9	HRS	2	++	nd

[a] HRS, hard red spring; HRW, hard red winter; HWS, hard white spring; SWS, soft white spring; SWW, soft white winter; and SWWC, soft white winter club.

[b] USDA estimates for Washington state obtained at http://www.nass.usda.gov/wa/whtvar02.pdf for each market class.

[c] Overall quality, where − designates lowest quality and +++ designates highest quality. Quality includes grain hardness, test weight, ash content, flour color, elasticity, loaf or cookie volume, and protein content, and varies according to market class (B. Carter, D. Engle, personal communication).

[d] Moderate to strong resistance to CB, common bunt; CS, Cephalosporium stripe; DB, dwarf bunt; DFR, dryland foot rot; FS, flag smut; HF, Hessian fly; LR, leaf rust; PM, powdery mildew; SE, sharp eyespot; SFR, strawbreaker foot rot; SM, snow mold; SR, stem rust; YR, yellow rust (stripe rust); and nd, no data.

[e] Formerly OR939526.

scribed in Landa et al. (2002a). Batches of ∼100 seeds were shaken in 0.5 ml of 1% methyl cellulose containing 10^7 colony forming units (CFU) of freshly plated bacteria, or in methyl cellulose without bacteria (control) until the seeds did not adhere to each other (McSpadden Gardener et al., 2001). Seeds were planted within 4 h of treatment.

2.2. Cultivation of plants

Sieved non-agricultural, non-pasteurized soil obtained from a site near Quincy, Washington (Raaijmakers et al., 1997) was used in all the experiments. This soil, called Quincy virgin, is a sandy loam that lacks natural suppressive activity, and has been used extensively as a standard soil in previous studies (Landa et al., 2003; Raaijmakers and Weller, 1998, 2001). The physical, chemical, and microbial composition of Quincy virgin soil have been described (Raaijmakers et al., 1997).

To minimize the impact of soil status and environment on root development, we more rigorously standardized planting, growth and watering regimens developed previously (Raaijmakers and Weller, 1998). Six-inch plastic cones (Stuewe & Sons, Corvallis, OR) were plugged with cotton and filled with approximately 70 ml of dry soil. To facilitate root harvest, a longitudinal cut was made in each cone using a utility knife and the cut was closed with duct tape before use. The soil in each cone was drenched with 50 ml of metalaxyl (75 mg/ L a.i., Novartis, Greensboro, NC) to control *Pythium* and other oomycetes at the start of planting. Plants were grown in a greenhouse at 15 ± 1°C with 12 h of daily supplemental lighting (66–90 μmol m^{-2} s^{-1}), and rotated on the bench every three to four days. To insure adequate moisture during germination, the cones were kept under plastic until hypocotyl emergence, usually for three days. Plants were subsequently uncovered for the duration of the experiment. Each cone received about 10 ml of distilled water every two to four days as needed,

and the water was supplemented with Miracle-Gro (0.94 g/L, Scotts, Port Washington, NY) at every second watering. Water was withheld for one to two days prior to harvest so that the roots could be removed from the soil without breakage.

In initial experiments, 27 cultivars were sown over three consecutive days (8–9 cultivars per day plus a standard, Penawawa), with four replicates per cultivar per treatment, then harvested at 14 dpi. The experiments were repeated for 16 of the cultivars, with six replicates per cultivar per treatment. Five cultivars (Edwin, Finley, Lambert, Penawawa, and Stephens) were selected to examine levels of root colonization at 4 and 7 dpi. For 4-day root colonization experiments, 4 treated seeds (Edwin, Lambert, and Penawawa only) were sown individually in 6-in. cones (four replicates per cultivar per bacterial treatment). Alternatively, 6–8 seeds were sown in each of two 7.5-cm square pots as described (Landa et al., 2002a,b), and two plants were sampled from each pot. Four-day-old plants received no water prior to harvest. For 7-day root colonization experiments, seeds were grown in pots and sampled as described above. Plants were watered with Miracle-Gro solution after four days. The 7-day experiment was repeated to generate a total of eight samples per cultivar.

2.3. Population densities of extractable bacteria

Extractable Q8r1-96 and Q2-87 from treated seeds or root samples were quantified using the 96-well dilution endpoint assay, described below (Landa et al., 2002a; McSpadden Gardener et al., 2001). Total culturable heterotrophic bacteria were also measured in the root extracts to monitor the natural state of the soil used throughout the experiments. Roots were excised at the base of the stem, shaken free of excess soil, and suspended in 10 ml of autoclaved distilled water. Bacteria were extracted from roots by 1 min of agitation using a vortex mixer, followed by 1 min of sonication. Each extract was serially diluted (100 μl of root extract plus 200 μl of water) up to 16 times in a microtiter plate. Fifty microliters of each dilution was transferred to microtiter plates containing 200 μl per well of either 1:10 tryptic soy broth (TSB) supplemented with 100 μg/ml cycloheximide (for total heterotrophic bacteria, including the introduced *P. fluorescens*) or 1:3 KMB supplemented with 40 μg/ml ampicillin, 13 μg/ml chloramphenicol, 100 μg/ml cycloheximide, and 100 μg/ml rifampicin (for introduced Rif$^+$ *P. fluorescens* only). Bacterial density was determined at 600 nm absorbance after 48 h for TSB cultures and 72–74 h for KMB cultures essentially as described previously (Landa et al., 2002a). Population densities were calculated from the most dilute (endpoint) sample in the dilution series that showed bacterial growth, specifically, samples having absorbance readings of $\geqslant 0.012$ absorbance units above background. Population densities were calculated as the log of colony forming units of Rif$^+$ or total bacteria per gram fresh weight of root (log(CFU g^{-1})).

To compare the uniformity of seed treatments and viability of the *P. fluorescens* strains on seeds, duplicate batches of 20 seeds from each treatment were extracted and cultured as described above within 24 h of coating.

To authenticate the genotypes of Rif$^+$ bacteria in root and seed extracts, two randomly selected endpoint samples from each treatment were subjected to PCR using primers BPF2 and BPR4 specific for the *P. fluorescens* *phl*D gene, which encodes a chalcone synthase-like protein of the 2,4-DAPG biosynthetic locus (Bangera and Thomashow, 1999). The resulting PCR products were cleaved with *Hae*III restriction enzyme, partitioned on agarose, and compared to RFLP patterns of the original strains (McSpadden Gardener et al., 2001). Following extraction and titration and prior to scanning, roots were stored at 4 °C.

2.4. Root scans

Root fresh weights and root scans were obtained within 24 h of extraction and dilution. Roots were scanned in custom-made 20×20 cm glass trays using a HP ScanJet 5370C (Hewlett Packard, Palo Alto, CA) or Epson Expression 1680 (Long Beach, CA) at no more than $1\times$ magnification using a computerized pixel-based program WinRHIZO 5.0 (Regent Instruments, Quebec, Canada). Average root diameter (mm), total root length (cm), surface area (cm^2), and total root volume (cm^3) were quantified using WinRHIZO algorithms. Total lengths of lateral roots (average diameter $\leqslant 0.5$ mm) and seminal roots (average diameter > 0.5–1.0 mm) were also quantified using WinRHIZO.

2.5. Statistical analyses

Bacterial population densities or root morphometric values for each bacterial isolate and cultivar were compared by general analysis of variance, and mean comparisons among treatments were performed using Fisher's protected least significant difference test at $P = 0.05$ (SAS GLM procedure, Version 8; SAS Institute, Cary, NC). Population densities were expressed as the mean CFU of Rif$^+$ bacteria per gram of root (for Q8r1-96:Q2-87 population ratios), or as the mean log CFU of Rif$^+$ bacteria per gram of root (for all other comparisons). Duplicate experiments were analyzed independently to determine if colonization responses were reproducible, and Bartlett's *t* test for equal variances was applied to determine whether data from duplicate experiments could be combined. A principal component analysis was performed for both bacterial isolates using four root architecture variables (total length, surface

area, average diameter, and fresh weight), two bacterial colonization variables [log(CFU g^{-1}) and log(CFU cm^{-1})], and the SAS FACTOR procedure. This analysis produced a set of variables that were linear combinations of the original variables. The new variables were independent of each other and were ranked according to the amount of variation for which they accounted. After initial factor extraction, an orthogonal varimax rotation was used to estimate the factor loadings.

3. Results

3.1. Wheat roots support higher levels of Q8r1-96 as compared to Q2-87

PNW wheat cultivars varied in extent to which they established and maintained interactions with two PNW *P. fluorescens* isolates, Q2-87 and Q8r1-96. At 14 dpi, mean rhizosphere populations of Q8r1-96 and Q2-87 for all 27 cultivars were log 7.96 ± 0.50 (1.7×10^8 CFU g^{-1}) and log 7.39 ± 0.48 (4.2×10^7 CFU g^{-1}), respectively. The population density of Q8r1-96 was significantly ($P < 0.041$) higher than that of Q2-87 for seven of the 27 cultivars. These results are presented in Fig. 1 as the ratio of the mean population of Q8r1-96 to that of Q2-87, where ratios were >1.0 for all cultivars but Bruehl, Macon, Residence, and Rod. Consistent with previous studies, a preference ($P = 0.001$–0.004) for Q8r1-96 was observed for Penawawa in all of our experiments. Culturable rhizosphere population sizes of strain Q8r1-96 ranged from log 7.2 (for Finley and

Macon) to log 8.8 (Buchanan, Tubbs), whereas those of strain Q2-87 ranged from log 6.5 (Finley, Scarlet) to log 8.2 (Buchanan, Coda). None of the cultivars sustained significantly ($P \geqslant 0.05$) higher levels of Q2-87 than Q8r1-96.

The population densities of Rif$^+$ bacteria on treated seeds ranged from 300 to 700 CFU per seed (average 500 CFU/seed) depending upon the experiment, and did not vary significantly ($P \geqslant 0.05$) between the two strains in any of the experiments.

*Hae*III cleavage of the *phl*D PCR product from Q8r1-96 resulted in fragments of 250, 290, and 330 bp in size and typical of D genotypes, whereas the Q2-87 *phl*D PCR product yielded *Hae*III restriction fragments of 170 and 340 bp, characteristic of the B genotype (McSpadden Gardener et al., 2001). Extracts of non-treated (control) roots did not yield *phl*D PCR products. Strains Q2-87 and Q8r1-96 from plate stocks served as positive controls.

Population densities of total heterotrophic bacteria were about 6- to 10-fold higher than those of the *P. fluorescens* isolates and no significant ($P \geqslant 0.05$) difference was observed in Q8r1-96- or Q2-87-treated roots (data not shown).

3.2. Cultivar variation in ability to support colonization by Q8r1-96 and Q2-87

The cultivars differed in ability to support rhizosphere colonization by each of the isolates, as shown in Fig. 1. Buchanan and Coda sustained relatively high rhizosphere populations of >log 8 (1.6×10^8 to 6.8×10^8
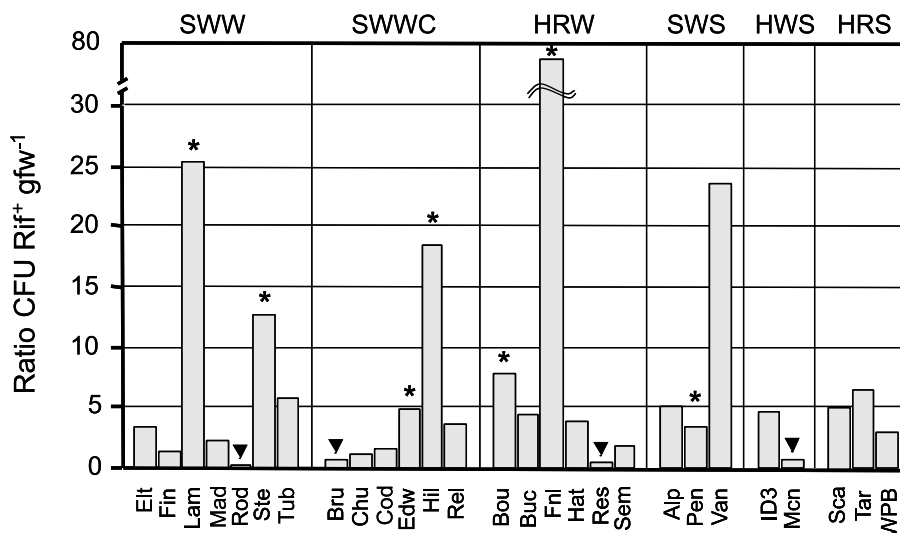

Fig. 1. Ratios of the rhizosphere populations (mean CFU per gram fresh root) of Q8r1-96 to those of Q2-87 for each of 27 PNW wheat cultivars at 14 days post-inoculation (dpi). Asterisks indicate cultivars for which population densities (CFU per gram of root) of Q8r1-96 are significantly ($P < 0.05$) greater than those of Q2-87 using combined data from two experiments. Black arrowheads designate cultivars that supported higher mean population densities of Q2-87 as compared to Q8r1-96. SWW, soft white winter; SWWC, soft white winter club; HRW, hard red winter; SWS, soft white spring; HWS, hard white spring; and HRS, hard red spring.

CFU g^{-1}) for both Q8r1-96 and Q2-87, whereas Scarlet supported the lowest levels at log 6.5 and log 7.2 (3.4×10^6 and 1.7×10^7 CFU g^{-1}) for Q2-87 and Q8r1-96, respectively. Finley showed the greatest differential colonization; the population density of Q8r1-96 was 77-fold greater than that of Q2-87 for this cultivar.

In the initial set of assays, the following sixteen cultivars sustained higher mean rhizosphere levels of Q8r1-96 as compared to Q2-87 at 14 dpi: Boundary, Buchanan, Chukar, Edwin, Eltan, Finley, Hatton, Hiller, ID377S, Lambert, Penawawa, Semper, Stephens, Tara, Tubbs, and Vanna (Fig. 1). In a second set of experiments that used more replicates, Boundary, Edwin, Finley, Hiller, Lambert, Penawawa, and Stephens sustained significantly ($P < 0.05$) higher levels of root-associated Q8r1-96 as compared to Q2-87 (Fig. 2), ranging from a 5-fold difference for Edwin to the 77-fold difference for Finley. In contrast, only Vanna showed differential root colonization in the first but not the second experiment (Figs. 1 and 2).

3.3. Differential root colonization can be observed at 7 dpi

Five of the seven cultivars that showed differential root colonization at 14 dpi were evaluated at 4 and 7 days (Fig. 3). At 4 dpi, rhizosphere population densities of both Q2-87 and Q8r1-96 were similar, indicating that colonization initially occurred to the same extent for both strains. Furthermore, population densities of Q8r1-96 were nearly the same at 4, 7, and 14 days; colonization by this isolate occurred rapidly and was sustained over the two-week interval. Differential colonization by the isolates was not observed at day 4.

In contrast to Q8r1-96, population densities of Q2-87 declined significantly ($P < 0.05$) by day 7 for Edwin, Finley, Penawawa, and Stephens (Fig. 3). Whether this preferential colonization is the result of mechanisms active against Q2-87 or supportive of Q8r1-96 remains to be determined.

The type of growth container (pots or cones) had no significant ($P \geqslant 0.05$) effect on the extent of root colo-
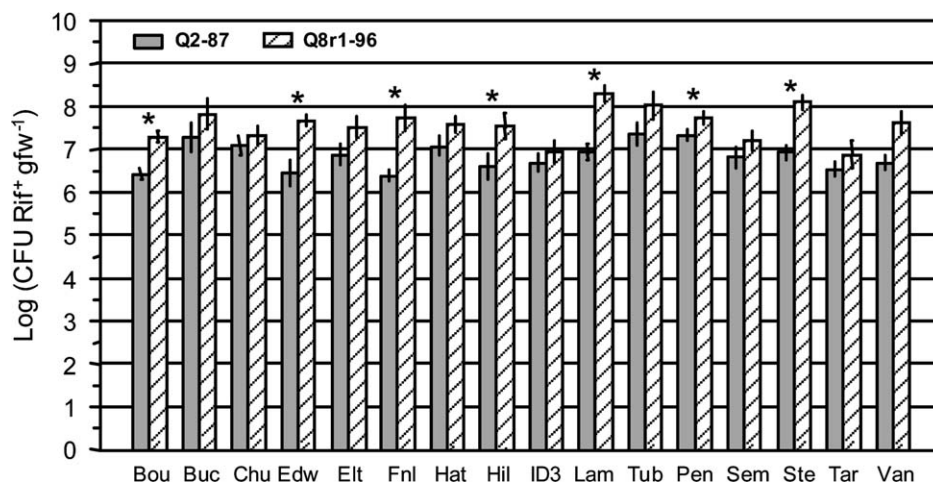

Fig. 2. Rhizosphere populations of culturable Rif$^+$ bacteria in selected PNW wheat cultivars. Asterisks indicate cultivars showing significant ($P < 0.05$) differences in log population values for Q8r1-96 and Q2-87 using combined data from two experiments. Bars represent standard error.
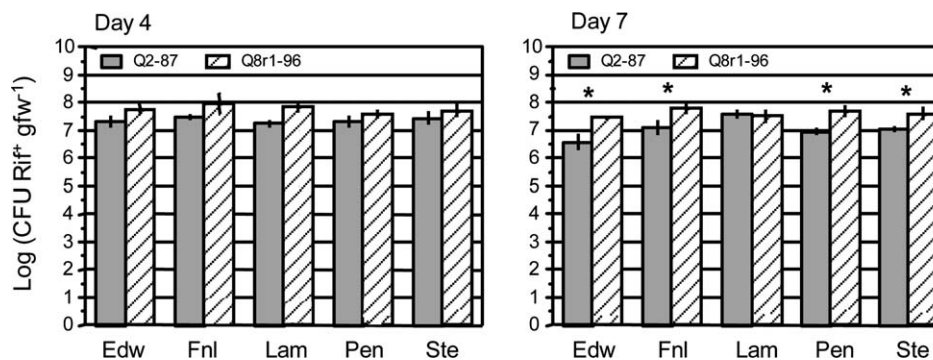

Fig. 3. Rhizosphere populations of culturable Rif$^+$ bacteria 4 days and 7 days after treatment with *P. fluorescens* Q2-87 (gray) or Q8r1-96 (hatched). Asterisks indicate cultivars showing significant ($P < 0.05$) differences in log population values for Q8r1-96 and Q2-87. Bars represent standard error.

nization by either bacterial isolate. We calculated that 12 doublings would account for rhizosphere population densities of $2–3 \times 10^6$ CFU per root, assuming that each seed carried 500 viable CFU. This increase could be a high estimate if a significant portion of the $10^4–10^5$ CFU used to coat each seed was viable but not extractable and/or culturable in our assays.

3.4. Cultivar-dependent differences in root development and morphometrics

With a few exceptions, the cultivars exhibited similar patterns of root growth and development, exemplified by Finley in Fig. 4. At 4 dpi, the primary root and two seminal roots had emerged and elongated. By 7 days, these had grown to the bottom of the cone, and additional seminal roots were present. At 14 days, seminal root development had progressed, and extensive formation of lateral roots was evident. The presence of brown, dried root segments in the soil at 14 dpi indicated that root die-back was occurring, particularly for Boundary (data not shown), but the extent of die-back was not quantified.

The pixel-counting program WinRHIZO was used to evaluate four morphometric variables, average diameter, total length, surface area, and total volume, from scans of the roots. Roots sampled at 4 dpi were not distinguishable with respect to any of the variables. As early as 7 dpi, Q8r1-96 treatment was correlated with higher ($P < 0.05$) average root diameter and total root length for Edwin, Finley, Lambert, and Stephens. In the former three, the correlation was seen at 7 dpi but not at 14 dpi. At 14 dpi, the cultivars displayed more diverse root architecture, and both positive and negative cor-

relations between morphometric variables and Q8r1-96 treatment were noted. When all 27 cultivars were considered together, average root diameter was positively and significantly ($r > 0.42$; $P < 0.03$) correlated with Q8r1-96 population densities, whereas total length was generally reduced in Q8r1-96-treated roots. An increase in average root diameter can result if lateral root formation and growth is inhibited, a condition that has been observed during pathogen infection (T. Paulitz, personal communication).

The morphometric analyses indicated that no single variable was associated with differential colonization by Q8r1-96 in non-agricultural Quincy soil. However, some cultivars displayed significant differences for specific morphometric values in pairwise comparisons between the two bacterial treatments. In Stephens, Q8r1-96 treatment was correlated with higher root volume and surface area at 14 dpi, as compared to the control or Q2-87 treatments. Exceptions to the inhibitory effect of Q8r1-96 were seen for ID377S, for which root volume, length, and surface area were higher than in Q2-87-treated roots, and Finley and Stephens, for which root weights were positively and significantly ($r > 0.42$; $P < 0.03$) correlated with Q8r1-96 population densities [log(CFU g^{-1}) and log(CFU cm^{-1})].

Lateral roots were usually $\leqslant 0.5$ mm in average diameter, and seminal roots were typically $>0.5–1.0$ mm in average diameter. To estimate the mass ratios of lateral to seminal roots for each root sample, we divided the total length of roots in the first diameter category with that of roots in the second category. Lateral-to-seminal root ratios ranged from ~4.5 for Q8r1-treated Alpowa and Vanna to 0.7 for Q8r1-treated Stephens. As compared to Q8r1-96, Q2-87 treatment was not associated
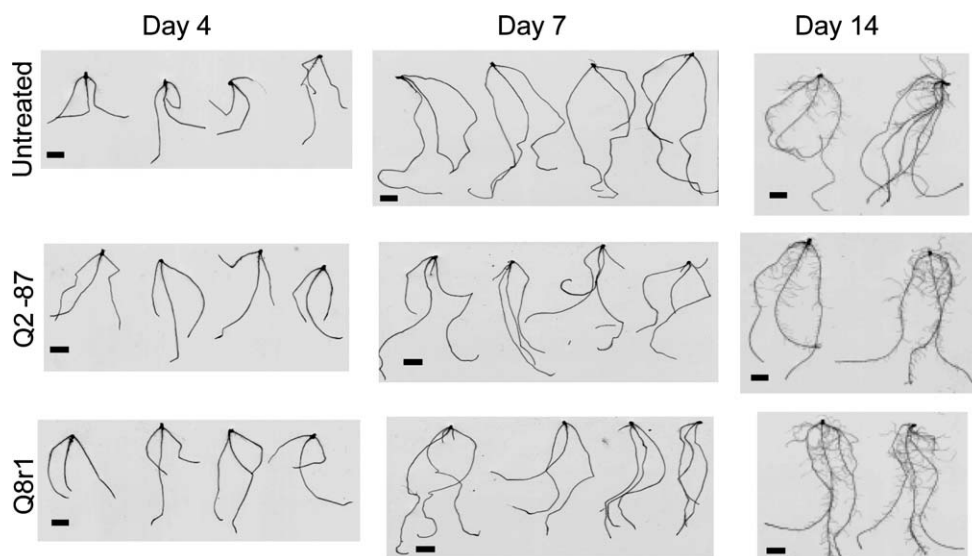

Fig. 4. Scans of roots of cv. Finley germinated from untreated, Q2-87-treated, and Q8r1-96-treated seeds. Roots were harvested at 4, 7, and 14 dpi. Bar = 1 cm.

with as wide a range of ratio values. Finally, the ratios for Q8r1-96- and Q2-87-treated roots were significantly ($P < 0.05$) different for Residence, Stephens, and Tara, but not for most of the differentially colonized cultivars.

3.5. Principle component (factor) analysis

The trend of significant differences in root colonization and morphometrics for the two bacterial treatments prompted us to perform a principle component analysis. We sought to determine the global effects of bacterial treatment on all of the root variables. The factor analysis was performed using values for four root-related variables (total length, total surface area, average diameter, and fresh weight), and two bacterial colonization variables [$\log(\text{CFU g}^{-1})$ or $\log(\text{CFU cm}^{-1})$] for two strains of *P. fluorescens* and 27 wheat cultivars (Table 2). Factors 1, 2, and 3 accounted for at least 94.8% of the total variance, whereas the variation attributed to Factors 4, 5, and 6 was marginal (data not shown). Therefore, only the first three factors were extracted from the considered variables and used to generate the eigenvalues shown in Table 2. Factors 1 and 3 were a combination of all root architecture-associated variables used in the analysis, and the corresponding values in the eigenvectors for each variable were used to interpret the significance of the factors. Factor 1 was dominated by high positive weights (>0.82) for total length, total surface area, and root weight; Factor 3 was dominated by high positive weight (>0.97) for average root diameter. Factor 1 can be thought of as a measure of root biomass, and together, Factors 1 and 3 can be considered as the amount of root accessible to colonizing bacteria and/or as the amount of nutrients provided.

Factor 2 was dominated by high positive weights (>0.99) for population densities of root-associated bacteria normalized to either root weight or root length, and represented the degree of root colonization by each of the two *P. fluorescens* strains.
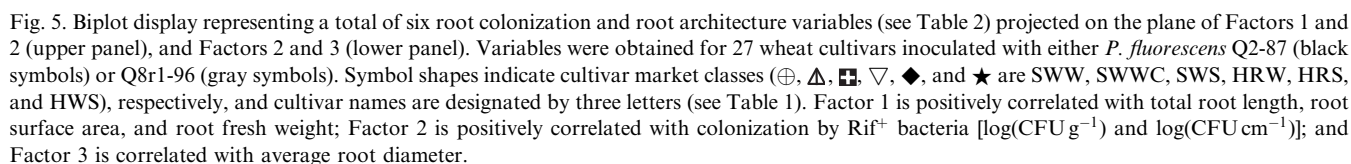
Distribution of the cultivars with respect to root colonization and root architecture is shown in Fig. 5. In the upper panel, Factor 2 is displayed relative to Factor 1, where the position of the icon from left to right along the X axis indicates increasing values for the root variables, and position along the Y axis from bottom to top indicates increasing values for bacterial population densities. Similarly, Factor 2 is plotted with respect to Factor 3 in the lower panel. Cultivars having the highest population densities of root-colonizing bacteria fell above the X axis in either Quadrants A or B.

Strain Q8r1-96 colonized the rhizosphere of most cultivars to a greater extent than did Q2-87, indicated by the occurrence of gray icons in Quadrants A and B and black icons in Quadrants C and D (Fig. 5). Residence was an exception to this trend. In general, Q8r1-96 had a negative effect on root biomass as compared to Q2-87, although it had significant positive effects on ID377S and Finley (Fig. 5, upper panel). Macon and Stephens had higher average root diameter values (Fig. 5, lower panel), and Alpowa and Boundary had lower values than other cultivars. The roots of Alpowa and Boundary were visibly less robust, and the factor analysis suggests that root diameter is a major variable for this observation.

The factor analysis supported our statistical analyses of root scan data, and substantiated visual observations of the effects of Q8r1-96 on roots. It also revealed new relationships, including the positive effect of Q8r1-96 on root weights of Hiller, Coda and Finch, and the negative effect of Q2-87 on the average root diameter of Chukar. Finally, Coda was distinguished as having the least differential with respect to either root biomass or average diameter for either bacterial treatment.

4. Discussion

We hypothesize that wheat–*Pseudomonas* rhizosphere interactions are governed in part by host-dependent factors, and that host traits and genes have direct roles in the persistence of specific strains of *P. fluorescens* in the wheat rhizosphere. Such genes can be identified using classical genetics, reverse genetics, or genomics approaches. As a first step to identifying useful genetic variation, we sought to (1) determine whether PNW wheat cultivars vary in their responses to PNW biocontrol strains Q8r1-96 and Q2-87, (2) identify cultivars that support higher rhizosphere population densities of one strain as compared to the other, and (3) determine

Table 2
Eigenvectors and eigenvalues of principal components derived from four root architecture-related variables[a] and two rhizosphere colonization variables[b] used to characterize the effect of wheat cultivar on rhizosphere colonization by *P. fluorescens* Q2-87 and Q8r1-96

Variable	Factor loadings		
	Factor 1	Factor 2	Factor 3
Avg diam (mm)	0.084	0.093	0.968*
Log(CFU/cm)	−0.031	0.991*	0.111
Log(CFU/g)	−0.018	0.999*	0.001
Surface area (mm²)	0.968*c	0.043	0.016
Length (cm)	0.816*	−0.005	−0.543
Wt (g)	0.865*	−0.113	0.227
Eigenvalues	2.41	2.00	1.27
Cum. expl. variance (%)[d]	40.16	73.54	94.78

[a] Avg diam (mm), total surface area (mm²), total length (cm), and avg fresh weight (g).
[b] Log(CFU/cm), log(CFU/g).
[c] Asterisk = values of variables dominating principal components F1, F2, and F3.
[d] Cumulative explained variance.

Fig. 5. Biplot display representing a total of six root colonization and root architecture variables (see Table 2) projected on the plane of Factors 1 and 2 (upper panel), and Factors 2 and 3 (lower panel). Variables were obtained for 27 wheat cultivars inoculated with either *P. fluorescens* Q2-87 (black symbols) or Q8r1-96 (gray symbols). Symbol shapes indicate cultivar market classes ($\oplus$, $\triangle$, $\boxplus$, $\triangledown$, $\blacklozenge$, and $\bigstar$ are SWW, SWWC, SWS, HRW, HRS, and HWS), respectively, and cultivar names are designated by three letters (see Table 1). Factor 1 is positively correlated with total root length, root surface area, and root fresh weight; Factor 2 is positively correlated with colonization by Rif$^+$ bacteria [log(CFU g^{-1}) and log(CFU cm^{-1})]; and Factor 3 is correlated with average root diameter.

whether aggressive root colonization is correlated to changes in specific root morphological variables.

Pseudomonas fluorescens strain Q8r1-96 was found to be a more effective and persistent rhizosphere colonizer than strain Q2-87 for most of the 27 PNW wheat cultivars tested in this study, suggesting that its superior colonizing ability is a phenomenon that supersedes cultivar variation. The aggressiveness of Q8r1-96 was observed in two additional experiments with a second seed lot (summer/fall 2002) of Finley, Lambert, and Stephens (P. Okubara, unpublished data). Although not all cultivars were tested, results from the above three suggest that differential colonization by Q8r1-96 was independent of seed lot. Finley showed the highest ratio of root-associated Q8r1-96 to Q2-87 (Fig. 2). In four separate experiments (including two done with the second seed lot), this ratio was 102, 7.6, 5.6, and 3.1, respectively, giving an overall ratio of 6-fold. We do not know why the ratio from our first experiment was un-

usually high, but the variation indicates the need for multiple independent tests.

Preferential colonization by Q8r1-96 was not attributable to better viability on the seeds of any of the cultivars. Likewise, differences in early colonization dynamics did not appear to be a factor, as the population densities of both strains were very similar at 4 dpi. However, colonization prior to 4 dpi has not yet been examined, and small differences in the timing of establishment of bacterial populations at the onset of the host interaction will likely impact 2,4-DAPG production, microbe–host signaling, and other processes critical to later stages of the interaction. Rapid establishment has been reported for aggressive rhizosphere colonizers (Adams and Kloepper, 2002), but additional processes that impact rhizosphere persistence appear to be expressed in Q8r1-96. In contrast, Q2-87 showed a wider range of rhizosphere population densities at 14 dpi, and its colonization was found to be more sensitive to host

genotype. While the aggressiveness of Q8r1-96 might be primarily determined by bacterial traits, the activity of Q2-87 appears to be mediated by host traits as well.

We have observed that the competency of wheat cultivars to undergo root colonization varies with the developmental stage of the host. Seven cultivars maintained high rhizosphere levels of Q8r1-96 at 14 dpi, whereas populations of Q2-87 declined significantly within this time frame. Four cultivars (of five tested) exhibited this differential response at 7 dpi but not at 4 dpi. Whether this response is the result of a host-mediated mechanism active against Q2-87 or supportive of Q8r1-96 remains to be determined.

The more rapid increase in total root length observed in Q8r1-96-treated roots as compared to Q2-87-treated roots at 7 dpi could indicate that Q8r1-96 exerts an earlier or stronger plant growth-promoting effect than does Q2-87. By 14 dpi, however, roots that underwent colonization by Q8r1-96 exhibited changes in morphology associated with pathogen and abiotic stress damage, specifically increased root diameter and decreased total length. Possible inducers of these morphological changes include the phytohormones methyl jasmonate and ethylene, both of which are known to exert developmental and stress-induced effects on roots (Ellis et al., 2002). We also noted that Q8r1-96 had a visible negative effect on root robustness for some of the cultivars, confirming other reports that the host plant can sustain a penalty for root colonization in absence of a pathogen (Fujimoto et al., 1995; Persello-Cartieaux et al., 2001). Increased production and/or accumulation of 2,4-DAPG by Q8r1-96 between 7 and 14 dpi might account for the inhibitory effect of Q8r1-96. Cultivar Stephens was unusual in its response to the biocontrol strains; Q8r1-96 significantly increased root weight, and Q2-87 appeared to have a positive effect on root volume and surface area. It is intriguing to consider that both strains could be exerting plant growth-promoting effects on Stephens, while only Q8r1-96 colonizes aggressively. Average root weights of ID377S and Finley also increased with Q8r1-96 treatment. In such cultivars, the apparent growth-promoting effect of Q8r1-96 is a conundrum, and might point to cultivar-specific tolerance to 2,4-DAPG and/or to other microbial interactions taking place in Quincy virgin soil.

Our analyses indicated that lateral root development was not correlated to preferential colonization by Q8r1-96. The degree of lateral branching can quantified using the *forks* function of WinRHIZO, but in our experiments, accurate quantification was complicated by frequent overlapping of the roots. There was no apparent correlation between Q8r1-96 root colonization and the extent of lateral root branching from visual comparisons of scanned images of the seven differentially colonized cultivars and nine other cultivars. Only Stephens showed an effect of bacterial treatment on the lateral to seminal

root ratio. Lateral root emergence is probably critical at very early stages in the host–microbe interaction. Neither was root growth rate found be significant; in experiments with untreated seedlings grown in Quincy virgin soil over a 10-day period, all cultivars exhibited similar rates of increase in root length, with Edwin and Semper having the highest rates, and Stephens and Tubbs the lowest (data not shown).

Although none of the quantitative root variables examined in this study was consistently associated with preferential colonization, recent studies with *Arabidopsis* mutants indicate that root hair development, and the plant morphogenetic hormones ethylene and cytokinin are important in *Pseudomonas*–root interactions (O'Callaghan et al., 2001; Persello-Cartieaux et al., 2001). Reverse genetics can readily be carried out in *Arabidopsis* to identify host pathways but this approach is complicated by the larger genome, higher ploidy, and longer generation time of hexaploid wheat.

Our data support previous observations that wheat genotype plays a role in disease suppression in the field, and impacts the types of microorganisms that dominate the wheat rhizosphere. Prior cultivation of orchard soils with wheat cultivars Penawawa and Lewjain, but not Hill 81 or Madsen, resulted in suppression of apple replant disease, caused by *R. solani* AG-5 (Mazzola, 2002; Mazzola and Gu, 2000, 2002). The former two cultivars stimulated rhizosphere populations of *Pseudomonas putida* that were antagonistic to *R. solani* AG-5 (Mazzola and Gu, 2000). Neal et al. (1970) reported that substitution of a chromosome pair in the spring wheat cultivar Apex, having resistance to common root rot, with the corresponding chromosomes of the susceptible cultivar S-615 was correlated with qualitative and quantitative changes in rhizosphere microflora. In our study, six of the seven cultivars that consistently sustained higher populations of Q8r1-96 are winter wheats. Although host genetics might underlie the variation in root colonization, there is no common parental line in their pedigrees to indicate an obvious genetic basis for the differential response. In fact, Boundary, Edwin, and Finley are derived from different lineages.

The basis for species and cultivar specificity in host–rhizosphere microbial interactions may involve one or more processes that promote the establishment and maintenance of the PGPR, including export of nutrients (Cieslinksi et al., 1997; Rengel, 2002), and production of biochemical attractants and other signals (Hawes et al., 1998; Teplitski et al., 2000). Wheat cultivars Penawawa and Fielder differed in their capacity to support signaling between rhizosphere bacteria via the quorum-sensing compound *N*-acyl homoserine lactone (Pierson et al., 1998). Host tolerance to the metabolites released by PGPR might also be important.

One approach to dissecting cultivar- and strain-variable responses in wheat is to characterize PGPR inter-

actions in situ. Several laboratories have localized *P. fluorescens* on host roots with the aid of the non-invasive reporter Green Fluorescent Protein (GFP) from the jellyfish *Aequorea victoria* (Bloemberg et al., 2000; Dandurand et al., 1997; Normander et al., 1999). The formation and distribution of microcolonies were not affected by the production of antifungal phenazines (Dandurand et al., 1997), but this has not been examined for 2,4-DAPG. The rhizoplane distributions of GFP-tagged Q8r1-96 and Q2-87 on roots of Penawawa are in progress, but colony size and location have proven inherently difficult to quantify (O. Mavrodi, L. Thomashow, unpublished results).

Host processes and genes will likely reflect mechanisms by which biological control agents exert disease-suppressive effects in vitro and in planta (Liu et al., 1995; Maurhofer et al., 1994; Van Wees et al., 1997; Weller et al., 2002). With the view to identifying such processes and genes, we will continue to characterize cultivar variation with respect to 2,4-DAPG accumulation, and global changes in host gene expression that take place during interactions with the biocontrol strains. Understanding how the host governs initial interactions and whether it has a direct or indirect role in maintaining rhizosphere populations of one strain while having the opposite effect on populations of another will provide insights to the complex dynamics of biological control in the rhizosphere.

Acknowledgments

References

Adams, P.D., Kloepper, J.W., 2002. Effect of host genotype on indigenous bacterial endophytes of cotton (*Gossypium hirsutum* L.). Plant Soil 240, 181–189.

Bangera, M.G., Thomashow, L.S., 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. J. Bacteriol. 181, 3155–3163.

Becker, J.O., Cook, R.J., 1988. Role of siderophores in suppression of *Pythium* species and production of increased growth response of wheat by fluorescent pseudomonads. Phytopathology 78, 778–782.

Bloemberg, G.V., Wijfjes, A.H.M., Lamers, G.E.M., Stuurman, N., Lugtenberg, B.J.J., 2000. Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different autofluorescent proteins in the rhizosphere, new perspectives for studying microbial communities. Mol. Plant–Microbe Inter. 13, 1170–1176.

Cieslinksi, G., Van Rees, K.C.J., Szmigielska, A.M., Huang, P.M., 1997. Low molecular weight organic acids released from roots of durum wheat and flax into sterile nutrient solutions. J. Plant Nutrit. 20, 753–764.

Cook, R.J., Ownley, B.H., Zhang, H., Vakoch, D., 2000. Influence of paired-row spacing and fertilizer placement on yield and root diseases of direct-seeded wheat. Crop Sci. 40, 1079–1087.

Dandurand, L.M., Schotzko, D.J., Knudsen, G.R., 1997. Spatial patterns of rhizoplane populations of *Pseudomonas fluorescens*. Appl. Environ. Microbiol. 63, 3211–3217.

De Leij, F.A.A.M., Dixon-Hardy, J.E., Lynch, J.M., 2002. Effect of 2,4-diacetylphloroglucinol-producing and non-producing strains of *Pseudomonas fluorescens* on root development of pea seedlings in three different soil types and its effect on nodulation by *Rhizobium*. Biol. Fertil. Soils 35, 114–121.

Ellis, C., Karafyllidis, I., Wasternack, C., Turner, J.G., 2002. The Arabidopsis mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. Plant Cell 14, 1557–1566.

Fujimoto, D.K., Weller, D.M., Thomashow, L.S., 1995. Role of secondary metabolites in root disease suppression. In: Inderjit, Dakshini, K.M.M., Einhellig (Eds.), Allelopathy Organisms, Processes, and Applications. American Chemical Society, Washington, DC, pp. 330–347.

Hawes, M.C., Brigham, L.A., Wen, F., Woo, H.H., Zhu, Y., 1998. Function of root border cells in plant health, pioneers in the rhizosphere. Annu. Rev. Phytopathol. 36, 311–327.

Kim, D.-S., Cook, R.J., Weller, D.M., 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology 87, 551–558.

Landa, B.B., de Werd, H.A.E., McSpadden Gardener, B.B., Weller, D.M., 2002a. Comparison of three methods for monitoring populations of different genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere. Phytopathology 92, 129–137.

Landa, B.B., Mavrodi, D.M., Thomashow, L.S., Weller, D.M., 2003. Interactions between strains of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* in the rhizosphere of wheat. Phytopathology 93, 982–994.

Landa, B.B., Mavrodi, O.V., Raaijmakers, J.M., McSpadden Gardener, B.B., Thomashow, L.S., Weller, D.M., 2002b. Differential ability of genotypes of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* strains to colonize the roots of pea plants. Appl. Environ. Microbiol. 68, 3226–3237.

Latour, X., Corberand, T., Laguerre, G., Allard, F., Lemanceau, P., 1996. The composition of fluorescent pseudomonad populations associated with roots is influenced by plant and soil type. Appl. Environ. Microbiol. 62, 2449–2456.

Liljeroth, E., 1995. Comparison of early root cortical senescence between barley cultivars, *Triticum* species and other cereals. New Phytol. 130, 495–501.

Liu, L., Kloepper, J.W., Tuzun, S., 1995. Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopathology 85, 695–698.

Maurhofer, M., Hase, C., Meuwly, P., Métraux, J.-P., Défago, G., 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0, influence of the *gac* A gene and of pyoverdine production. Phythopathology 84, 139–146.

Mavrodi, D.V., Mavrodi, O.V., McSpadden Gardener, B.B., Landa, B.B., Weller, D.M., Thomashow, L.S., 2002. Identification of differences in genome content among phlD-positive *Pseudomonas fluorescens* strains by using PCR-based subtractive hybridization. Appl. Environ. Microbiol. 68, 5170–5176.

Mazzola, M., 2002. Mechanisms of natural soil suppressiveness to soilborne diseases. Antonie van Leeuwenhoek 81, 557–564.

Mazzola, M., Gu, Y.-H., 2000. Impact of wheat cultivation in microbial communities from replant soils and apple growth in greenhouse trials. Phytopathology 90, 114–119.

Mazzola, M., Gu, Y.-H., 2002. Wheat genotype-specific induction of soil microbial communities suppressive to disease incited by *Rhizoctonia solani* anastomosis group (AG)-5 and AG-8. Phytopathology 92, 1300–1307.

McSpadden Gardener, B.B., Mavrodi, D.V., Thomashow, L.S., Weller, D.M., 2001. A rapid polymerase chain reaction-based assay characterizing rhizosphere populations of 2,4-diacetylphloroglucinol-producing bacteria. Phytopathology. 91, 44–54.

McSpadden Gardener, B.B., Schroeder, K.L., Kalloger, S.E., Raaijmakers, J.M., Thomashow, L.S., Weller, D.M., 2000. Genotypic and phenotypic diversity of *phlD*-containing *Pseudomonas* strains isolated from the rhizosphere of wheat. Appl. Environ. Microbiol. 66, 1939–1946.

Neal, J.L., Atkinson, T.G., Larson, R.I., 1970. Changes in the rhizosphere of spring wheat induced by disomic substitution of a chromosome. Can. J. Microbiol. 16, 153–158.

Normander, B., Hendriksen, N.B., Nybroe, O., 1999. Green fluorescent protein-marked *Pseudomonas fluorescens*, localization, viability, and activity in the natural barley rhizosphere. Appl. Environ. Microbiol. 65, 4646–4651.

Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D., Défago, G., 2001. Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* bioncontrol strain CHA0 in the rhizosphere. Phytopathology 91, 873–881.

O'Callaghan, K.J., Dixon, R.A., Cocking, E.C., 2001. *Arabidopsis thaliana*: a model for studies of colonization by non-pathogenic and plant-growth-promoting rhizobacteria. Aust. J. Plant Physiol. 28, 975–982.

Persello-Cartieaux, F., David, P., Sarrobert, C., Thibaud, M.-C., Achouak, W., Robaglia, C., Nussaume, L., 2001. Utilization of mutants to analyze the interaction between *Arabidopsis thaliana* and its naturally root-associated *Pseudomonas*. Planta 212, 190–198.

Pierson, E.A., Wood, D.W., Cannon, J.A., Blachere, F.M., Pierson III, L.S., 1998. Interpopulation signaling via *N*-acyl-homoserine lactones among bacteria in the wheat rhizosphere. Mol. Plant–Microbe Interact. 11, 1078–1084.

Raaijmakers, J.M., Weller, D.M., 1998. Natural plant protection by 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. Mol. Plant–Microbe Interact. 11, 144–152.

Raaijmakers, J.M., Weller, D.M., 2001. Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: char-acterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. Appl. Environ. Microbiol. 67, 2545–2554.

Raaijmakers, J.M., Bonsall, R.F., Weller, D.M., 1999. Effect of population density of *Pseudomonas fluorescens* on production of 2,4-diacetylphloroglucinol in the rhizosphere of wheat. Phytopathology 89, 470–475.

Raaijmakers, J.M., Weller, D.M., Thomashow, L.S., 1997. Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. Appl. Environ. Microbiol. 63, 881–887.

Rengel, Z., 2002. Genetic control of root exudation. Plant Soil 245, 59–70.

Scott, P.R., 1981. Variation in host susceptibility. In: Asher, M.J.C., Shipton, P.J. (Eds.), Biology and Control of Take-all. Academic Press, London, pp. 219–236.

Simon, H.M., Smith, K.P., Dodsworth, J.A., Guenthner, B., Handelsman, J., Goodman, R.M., 2001. Influence of tomato genotype on growth of inoculated and indigenous bacteria in the spermosphere. Appl. Environ. Microbiol. 67, 514–520.

Smith, K.P., Goodman, R.M., 1999. Host variation for interactions with beneficial plant-associated microbes. Annu. Rev. Phytopathol. 37, 473–491.

Smith, K.P., Handelsman, J., Goodman, R.M., 1999. Genetic basis in plants for interactions with disease-suppressive bacteria. Proc. Natl. Acad. Sci. 96, 4786–4790.

Smith, J.D., Kidwell, K.K., Evans, M.A., Cook, R.J., Smiley, R.W., 2003a. Assesment of spring wheat genotypes for disease reaction to *Rhizoctonia solani* AG-8 in controlled environment and direct-seeded field evaluations. Crop Sci. 43, 694–700.

Smith, J.D., Kidwell, K.K., Evans, M.A., Cook, R.J., Smiley, R.W., 2003b. Evaluation of spring cereal grains and wild *Triticum* germplasm for resistance to *Rhizoctonia solani* AG-8. Crop Sci. 43, 701–709.

Teplitski, M., Robinson, J.B., Bauer, W.D., 2000. Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol. Plant–Microbe Interact. 13, 637–648.

Van Wees, S.C.M., Pieterse, C.M.J., Trijssenaar, A., Van t'Westende, Y.A.M., Hartog, F., Van Loon, L.C., 1997. Differential induction of systemic resistance in *Arabidopsis* by biocontrol bacteria. Mol. Plant–Microbe Inter. 10, 716–724.

Weller, D.M., Cook, R.J., 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73, 463–469.

Weller, D.M., Cook, R.J., 1986. Increased growth of wheat by seed treatments with fluorescent pseudomonads, and implications of *Pythium* control. Can. J. Plant Pathol. 8, 328–334.

Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu. Rev. Phytopathol. 40, 309–348.

Windels, C.E., 2000. Economic and social impacts of Fusarium head blight, changing farms and rural communities in the northern great plains. Phytopathology 90, 17–21.